Cytomegalovirus-specific T-cell dynamics in HIV infection
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Chapter 8

General Discussion
GENERAL DISCUSSION

Although often asymptomatic, cytomegalovirus (CMV) infection can lead to serious clinical complications and even death. Particularly in individuals who are immunocompromised or immunosuppressed, CMV reactivation or (re)infection can lead to overt disease. CD8⁺ and CD4⁺ T cells are playing a key role in the defence against viral infections such as CMV. Progression to disease has therefore been suggested to be caused by a defective immune response such as dysfunction of T cells or physical loss of virus-specific T cells. On the other hand, it has been hypothesised that a strong cellular immune response against CMV can also lead to pathology, characteristic of CMV end-organ disease. In this thesis, we describe a number of studies in which CMV-specific CD8⁺ and CD4⁺ T cell responses were analysed in detail in the natural course of human immunodeficiency virus (HIV)-1 infection, to identify immunological parameters that may determine why some patients progress to CMV end-organ disease. To this end, HIV-1-infected individuals selected from the “Amsterdam Cohort Studies on HIV-1 infection and AIDS among homosexual men”, who either remain long-term asymptomatic, or progress to AIDS with or without CMV end-organ disease were studied.

Dysfunction of CMV-specific CD8⁺ T cells in terms of IFNγ production despite a highly differentiated effector phenotype in progression to CMV end-organ disease

Since CD8⁺ T cells are important in defence against viral infections, we studied the CMV-specific CD8⁺ T-cell response in terms of number, function, and phenotype, using HLA-A2 tetrameric molecules presenting the immunodominant NLVPMVATV epitope to analyse CMV-specific CD8⁺ T cells in number. In terms of function, IFNγ production, after stimulation with peptide, or perforin and granzyme B double expression was used as a read-out. Staining with tetramers in combination with phenotypic markers determined differentiation characteristics of these cells. We showed that CMV-specific CD8⁺ T cells are abundantly present and increase during the course of HIV infection in individuals progressing to AIDS with CMV end-organ disease (i.e. AIDS-CMV; chapter 2). This differs from observations in progressors to AIDS-related non-Hodgkin lymphoma (AIDS-NHL) and progressors to AIDS where numbers of Epstein-Barr virus (EBV)-specific CD8⁺ T cells and HIV-specific CD8⁺ T cells, respectively, remained stable during follow-up [1,2]. In general, CMV-specific CD8⁺ T cells were found in higher numbers compared to HIV- and EBV-specific CD8⁺ T cells (chapter 7). CMV-specific IFNγ-producing CD8⁺ T-cell numbers were low in individuals progressing to AIDS-CMV, suggesting that CMV end-organ disease is associated with dysfunction rather than physical loss of CMV-specific CD8⁺ T cells. Also, EBV- and HIV-specific CD8⁺ T cells were shown to become progressively dysfunctional in progressors to AIDS-NHL and progressors to AIDS, respectively. In contrast, EBV- and HIV-specific CD8⁺ T cells were also shown to have an immature phenotype, whereas CMV-specific CD8⁺ T cells were observed to
be highly differentiated effector T cells with lack of CD27 expression and high levels of perforin and granzyme B co-expression (chapters 2, 3, and 7; [3-6]). Even in progressors to AIDS-CMV, perforin and granzyme B double expressing CD8+ T cells were abundantly present, and were also shown to be fully differentiated CD45RO+/-, CD27+, CCR7+ T cells (chapters 2 and 3). This suggests that the lack of IFNγ production is not due to a block in maturation, and that, in contrast to EBV- and HIV-specific T cells [7,8], CMV end-organ disease develops in the face of highly differentiated CMV-specific effector CD8+ T cells. The observation that IFNγ release and perforin and granzyme B expression - both potentially powerful antiviral mechanisms - are not linked, has been reported previously [9,10]. With CMV being a typical cytopathic virus [11], which have been reported to depend more on IFNγ and TNFα for control [12], the decreased IFNγ-producing capacity of the CMV-specific CD8+ T cells may well play a crucial role in progression to CMV end-organ disease.

Loss of CMV-specific cytokine-producing CD4+ T cells in progression to CMV end-organ disease

CD4+ help seems to be important in different stages of the CD8+ T-cell immune response (reviewed in [13,14]). In parallel with CD8+ T-cell measurements, we analysed the dynamics of CMV-specific IFNγ-producing CD4+ T cells after stimulation with CMV lysate (chapter 2). In HIV-infected individuals who progress to AIDS with CMV end-organ disease, these cells were lost in the year before onset of clinical disease, suggesting that CMV-specific IFNγ+CD4+ T cells play an important role in protection from CMV-associated disease. The importance of these IFNγ+CD4+ T cells has also been shown in transplantation patients, where acute symptomatic CMV-associated disease was only observed in patients who lacked an IFNγ+CD4+ T-cell response [15,16]. Possibly, these cells could play a role in the observed dysfunction of CTL (in terms of IFNγ), and in controlling CMV directly, through the antiviral effect of IFNγ. To elucidate the role of CMV-specific CD4+ T cells further, we continued to analyse the CD4+ T-cell response in terms of both IFNγ and IL-2 production, proliferation, and phenotype of cytokine-producing CMV-specific CD4+ T cells (chapter 5). Recent studies have described that HIV-specific IL-2-producing central memory (i.e. CCR7+CD45RA-) CD4+ T cells are associated with low load and long-term non-progression towards AIDS [17] and recovered partly after prolonged anti retroviral therapy (ART) [18]. Furthermore, Zaph et al [19] have shown that Leishmania major-specific IL-2-producing, central memory CD4+ T cells provided protection against disease in mice. The observed lack of CMV-specific IFNγ and/or IL-2-producing CD4+ T cells as well as their proliferative capacity during progression towards AIDS-CMV points to the importance of these cells in protection from disease. (Central) memory CD4+ T cells have been suggested to be IL-2-producing cells with proliferative properties that are able to differentiate to IFNγ-producing, effector (memory) CD4+ T cells that are less capable of proliferation. However, Harari et al [20] have recently published a report describing that memory (i.e. the ability to mount an accentuated response to antigen re-encounter) antigen-specific
CD4⁺ T cells express heterogeneity in both function and phenotype and depend strongly on antigen persistence and antigen load. In chronic CMV infection, memory CD4⁺ T cells were found to be of a mixed phenotype, namely both IFNγ- and IL-2-producing subsets expressing CCR7⁺/− CD45RA⁺/− phenotypes. This is in line with our observations that most CMV-specific CD4⁺ T cells produce IFNγ and/or some IL-2, and express a CD45RO⁺CD27⁻ phenotype as expected in infection with repetitive antigen exposure. During progression to AIDS-CMV, these cells shifted towards a fully differentiated CD45RO⁻CD27⁻ “effector” phenotype, probably associated with antigenic drive.

Of course one can never exclude the possibility that these CMV-specific CD4⁺ T cells have all migrated to the site(s) of infection. Moreover, it is highly likely that CMV-specific CD4⁺ T cells do go to the site(s) of infection, to play a “helper” role, as well as a direct role in controlling the virus. However, the absolute lack of CMV-specific cytokine-producing CD4⁺ T cells in progressors to CMV end-organ disease and the active CMV replication with high levels of CMV load in PBMC, suggests that loss of CMV-specific CD4⁺ T cells plays an important role.

CMV-specific HLA-DR3 tetrameric molecules detect CMV-specific CD4⁺ T cells in CMV infection

To measure CMV-specific CD4⁺ T cells directly ex vivo without in vitro manipulation, we developed HLA-DR3 tetrameric molecules specific for a single epitope. Using these novel MHC class II tetrameric molecules (chapter 4), we were able to detect CMV-specific CD4⁺ T cells in CMV-seropositive individuals in the context of HLA-DR3. One of our questions was whether, in individuals progressing to CMV end-organ disease, the CMV-specific CD4⁺ T cells were lost altogether, or whether they remained present but were rendered dysfunctional. Although we were able to detect HLA-DR3-restricted CMV-specific pp65 p511-522 CD4⁺ T cells early in infection in a few individuals, in general numbers were low and difficult to detect. Specific expansion was needed to confirm our direct ex vivo analyses. However, as proliferation of CD4⁺ T cells is affected - especially in progressors to AIDS-CMV - this complicates detection of CMV-specific CD4⁺ T cells using CMV-specific HLA-DR3 tetrameric molecules, making it difficult to answer the question regarding depletion or dysfunction with the current data. Future studies with additional epitopes, and/or other clinical settings - or methods to overcome impaired proliferation - could circumvent the issue of potentially difficult staining of virus-specific CD4⁺ T cells with class II tetrameric molecules, and provide an answer.

CMV load and CMV end-organ disease

During acute infection, for instance in transplantation patients, the virus multiplies and CMV load can be detected in PBMC. T-cell immunity is induced and the viral load decreases to undetectable levels [15]. In chronically infected, healthy individuals, CMV DNA levels in PBMC cannot be detected normally. In chronically infected transplant recipients on immunosuppressive therapy, however, CMV load can be
detected during reactivation or re-infection [16]. To study what happens to the virus in HIV-1-infected individuals, we measured CMV load levels in PBMC (chapter 2). In most HIV$^+$ individuals, CMV load was not detectable at the time points we selected for our longitudinal study. However, in progressors to AIDS-CMV, high CMV load was detected in the year before onset of symptoms, indicating that CMV load can be used as a marker for progression to CMV end-organ disease. Indeed, it has been shown previously that increased CMV load in plasma, whole blood or urine in advanced HIV-1-infected patients was a reliable marker for progression to AIDS as well as mortality, and that effective pre-emptive therapy against CMV could improve survival chances [21-23].

Besides CMV DNA levels in the blood, presence of CMV in the urine is a measure for persistent viral replication. In chapter 6, HIV-1-infected paediatric patients who are on highly active anti-retroviral therapy (HAART) were followed, and a proportion of these patient showed CMV shedding in their urine. Shedding is an indication that these individuals are not as successful in controlling the virus compared to non-shedding patients. Similar to HIV-1-infected adults who progress to AIDS-CMV (chapter 2), CMV-specific IFN$\gamma$-producing CD$^{8+}$ T cells were significantly lower in these HIV-1-infected CMV-shedding children compared to non-shedding patients (chapter 6). IFN$\gamma$-producing CD$^{4+}$ T-cell numbers were lower in CMV-shedding patients but this was not significant. Still, the fact that CMV-shedding individuals apparently are not successful in controlling viral replication indicates that these CMV-shedding patients are at higher risk of developing CMV end-organ disease. However, the patients are on HAART and the incidence of CMV end-organ disease in AIDS patients dropped dramatically since the introduction of HAART [24]. Komanduri et al [25] have shown an association between a decline in CMV-specific CD$^{4+}$ T-cell frequencies and the inability to sustain high levels of CMV-specific CD$^{8+}$ T cells in patients on potent combination antiretroviral therapy. Furthermore, patients on HAART with a recurrence of CMV retinitis do not show reconstitution of CMV-specific CD$^{4+}$ T cells, although these might be restored after anti-CMV therapy [26-28].

**CMV-dependent shaping of the cellular immune response**

CMV infection somehow induces T cells to fully differentiate to effector T cells during progression to CMV end-organ disease (chapters 3 and 5). Moreover, a number of studies have suggested that CMV is also the driving force in shaping the T-cell immune response in general [29,30]. These studies have shown that the CD$^{8+}$ T-cell population is enriched in effector CD$^{8+}$ CD$^{45RA^-CD27^+}$ T cells [31], and the CD$^{4+}$ T-cell subset in CD$^{4+}$CD$^{28^-}$ T cells [32]. We have shown that CMV shedding in the urine is implicated in further enrichment (compared to healthy CMV-seropositive individuals) of the effector CD$^{8+}$ T-cell pool in HIV-infected children (chapter 6). In addition, in subjects progressing to CMV end-organ disease who have higher occurrence of CMV-reactivation, also the CD$^{4+}$ CD$^{27^-CD28^-}$ T-cell pool is enlarged (chapter 3). The first paper to associate changes in T-cell subsets with age
and CMV status was published in 1999 [33,34]. Even though both age and CMV status influence the number of T cells, here they describe that increased numbers of CD4+CD28+ and CD8+CD28+ T cells are primarily associated with CMV status, and only secondarily with age. This is in line with the later studies, because CMV status influences CD8+ and CD4+ T-cell subset outgrowth both in adults and children. How CMV might shape the immune response is not clear yet. One explanation could be that the observed effect is due to large expansions of CMV-specific T cells, which express a relatively high differentiated phenotype. This highly differentiated phenotype is indeed observed in CMV-specific T cells. However, the magnitude of the observed effect makes it unlikely that CMV-specific T-cell expansions are solely responsible for the observed shaping of the immune response as a whole. Therefore, the explanation that CMV drives a bystander effect as well, contributing to the observed general outgrowth, is more likely.

Pathogenesis of CMV end-organ disease in HIV infection

Based on the results presented in this thesis and current literature, one could propose the following simplified model for CMV-related disease development in HIV infection (Figure 1). Infection with CMV in humans leads to persistent infection, usually without symptomatic disease. After primary CMV infection, CMV may establish latency but periodic reactivation is likely to occur. CMV reactivation or (re-)infection can lead to serious clinical complications, when it occurs in immunocompromised individuals. In HIV-infected individuals, especially in those who are progressing towards AIDS, CD4+ T-cell numbers decrease and their immune system appears to be chronically activated. CMV end-organ disease is a late-stage, AIDS-defining event and generally occurs when CD4+ T-cell numbers are below 50 CD4+ T cells/µl. In progressors to AIDS-CMV, CMV-specific CD4+ T cells can be detected initially. Substantially higher numbers of CMV-specific CD4+ T cells produce much more IFNγ with respect to IL-2 and some T cells produce both. The IFNγ-producing CMV-specific CD4+ T cells are mainly of the CD45RO'CD27- effector phenotype, in line with repetitive antigen exposure characteristic of CMV. During progression to CMV end-organ disease, these IFNγ-producing CD4+ T cells shift towards fully differentiated CD45RO'CD27+ "effector" T cells with lack of IL-2 production and proliferative capacity. Antigenic drive by CMV and thereby exhaustion of CMV-specific T cells might play an important role in HIV-infected individuals, since immune activation and exhaustion in general is a characteristic of HIV infection. Eventually the CMV-specific CD4+ T cells also fail to produce IFNγ. Lack of IL-2 may also play a role in causing insufficient help to sustain CMV-specific IFNγ-producing CD8+ T cells, which decreased during progression to CMV end-organ disease. The loss of these IFNγ-producing CD8+ (and probably CD4+) T cells leads to loss of control and CMV dissemination, as reflected by increased CMV DNA levels in PBMC. The many CMV-infected cells are prone to cell lysis not only because of the cytopathic properties of CMV, but also due to the killing by perforin and granzyme B expressing CMV-specific CD8+ T cells that are detected in large
quantities. This leads to the severe immunopathology, i.e. tissue destruction, characteristic of CMV end-organ disease.

![Diagram showing the interaction between CD4+ and CD8+ T cells in the context of CMV infection](image)

**Figure 1. Schematic representation of CMV-related disease development in HIV infection.**

A factor in progression to CMV end-organ disease that should not be excluded is CMV itself. Differences in strains of the virus are likely to influence progression to disease. CMV is a virus with a broad scala of immune evasion strategies. In theory the virus has all the genes to be able to remain invisible to the immune system (e.g. MHC I and II down modulation, cytokine homologues such as IL-10). However, cellular immunity does seem to play an important role in controlling infection and apparently, there is a balance in healthy individuals between the virus and its host.

**Future studies**

Most studies, including research described in this thesis, have studied the immune response directed against pp65, the lower matrix protein of CMV. However, as CMV expresses many proteins at different stages during infection, responses to other proteins may well play a role. For example, the immediate early antigen-1 (IE-1), another protein of CMV, has been reported to play an additional role to pp65. Furthermore, not many epitopes have been identified making the HLA-A2 NLVPMVATV T-cell response the most studied one to date. Nowadays, there are overlapping peptide pools for both pp65 and IE-1. It is likely that more epitopes will be identified through epitope mapping with these pools. Especially for CMV-specific CD4+ T-cell responses, more defined immunodominant epitopes should provide helpful tools to better understand the complexity of the immune response.
Furthermore, HLA-DR3 tetrameric molecules seem promising clinical tools that can be used to investigate HLA-DR3-restricted antigen-specific CD4⁺ T cells. MHC class II tetrameric molecules offer another way to detect CD4⁺ T cells in, for example, infectious disease studies. With the identification of more HLA-restricted CD4-dependent immunodominant T-cell epitopes, a greater number of epitope-specific class II tetrameric molecules can be developed to perform patient studies or vaccination trials in much more detail with inclusion of a more varied HLA-type. Finally, since CMV-specific CD4⁺ T cells seem to play an important role in addition to CD8⁺ T cells in progression to CMV end-organ disease, these class II tetrameric molecules could provide helpful tools in adoptive T-cell therapy, potentially allowing restoration of deficient responses in, for example, immunocompromised individuals such as HIV-infected patients.

REFERENCES


Chapter 8


